Effect of labetalol on adrenergic transmission in the rat anococcygeus muscle

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Labetalol has been reported to block α- and β-adrenoceptors in several tissues (Brittain & Levy, 1976). In the present study we have compared the α-adrenoceptor blocking actions of labetalol, prazosin and phentolamine in the rat anococcygeus muscle. Contractile responses of the rat anococcygeus muscle were recorded isometrically as described by Gillespie (1972). Mean vales were determined from at least 5 observations and are expressed ± s.e. mean.

Labetalol (10^{-5}M and 10^{-6}M) induced marked spontaneous activity which was abolished by phentolamine (5×10^{-6}M). After this spontaneous activity had disappeared (2-3 h), labetalol (10^{-5}M and 10^{-6}M) alone, or in the presence of nortriptyline (10^{-6}M), had no effect on responses to (-)-noradrenaline. In the presence of nortriptyline (10^{-6}M), phentolamine (10^{-6}M) and prazosin (10^{-8}M) inhibited responses to (-)-noradrenaline with pA_2 values of 6.76 and 8.86, respectively. Labetalol (10^{-6}M) did, however, inhibit responses to (-)-noradrenaline in the presence of guanethidine (6×10^{-6}M)); the pA_2 value being 6.76. Labetalol (10^{-6}M) abolished responses to field stimulation (1 ms, supramaximal voltage) at 0.1-2 Hz, and reduced responses at higher frequencies. In the presence of nortriptyline (10^{-6}M) this inhibitory effect of labetalol was significantly reduced. Guanethidine (6×10^{-6}M) abolished responses to field stimulation at 0.1-2 Hz and reduced responses at higher frequencies; this effect was partially reversed by nortriptyline (10^{-6}M). Both labetalol and guanethidine were thus less potent inhibitors of responses to field stimulation in the presence of nortriptyline. By contrast, all responses to field stimulation (0.1-40 Hz) were inhibited by phentolamine (10^{-6}M) and prazosin (10^{-7}M), in the presence of nortriptyline (10^{-6}M).

Maximum responses to tyramine in the absence of other drugs, and in the presence of 10^{-5}M and 10^{-6}M labetalol were 98% ± 6, 9% ± 9, and 61% ± 5, respectively, of the maximum response to (-)-noradrenaline. In the presence of phentolamine (10^{-6}M), responses to tyramine were abolished.

These results suggest that labetalol releases (-)-noradrenaline in the rat anococcygeus muscle. A depletion of noradrenaline stores or an impairment of release may explain why labetalol is a more potent inhibitor of responses to field stimulation and tyramine than of (-)-noradrenaline in this tissue.

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Factors influencing the inhibitory effectiveness of clonidine on adrenergic transmission: the relationship between clonidine-induced inhibition and the duration of interval between stimuli

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It has been reported earlier (Idowu & Zar, 1976) that clonidine inhibits the adrenergic motor transmission in the anococcygeus by presynaptic mechanisms. In the present investigation we have ascertained the effect of the duration of the interval between stimuli upon the clonidine-induced inhibition of the adrenergic transmission.

Isolated anococcygii from male rats were set up in 10ml organ baths between parallel platinum electrodes in Krebs-Henseleit solution at 37°C and contractions were recorded isometrically. For electrical field stimulation, 5 pulses of 1 ms duration at 10 Hz were delivered at supramaximal voltage, at variable intervals (3, 7.5, 15, 30, 60 s). The inhibitory effect of clonidine on adrenergic motor transmission was most marked at 60 s intervals and declined as the interval between the delivery of the 5-pulse trains decreased (Table 1). A possible explanation (Starke, 1972) for this relationship between clonidine-induced inhibition and the interval between stimuli might lie in a feed-back inhibition of transmitter release by endogenous noradrenaline (NA); with shorter intervals between stimuli, larger biophase concentrations of NA is to be expected and is likely to produce a greater degree of feed-back inhibition, thus masking the effectiveness of clonidine. This possibility is sup-

Table 1 The effect of duration of the interval between stimuli upon the inhibition by clonidine (3 nM)

Interval between stimuli (sec)	*% Inhibition after clonidine (Mean ± s.e.mean)*
60	69.6 ± 4.7
30	65.7 ± 4.5
15	55.8 ± 4.7
7.5	32.0 ± 5.7
3	−10.3 ± 2.9

The results are the means ± s.e.mean of 5 experiments on electrically evoked contractions (5 pulses, 1 ms, 10 Hz) of rat anococcygeus.

ported by our observation that the clonidine-induced inhibition bears an inverse relationship with the train-length; contractions evoked by short trains of stimuli were inhibited to a greater extent than those produced in response to longer trains (% inhibition ± s.e.mean at 10 Hz: 84.40 ± 7.8 with 2 pulses; 1.9 ± 4 with 40 pulses). However, other findings do not support this explanation; niether NA (10^{-9}M–10^{-6}M) nor tyramine (10^{-7}M–10^{-5}M) inhibited the transmission and clonidine-induced inhibition persisted unimpaired after treatment with cocaine (3×10^{-6}M). An alternative explanation is that the intraneuronal calcium levels determine the extent of clonidine-induced inhibition and that NA does not exert a feed-back inhibition in this issue.

Experiments involving the effect of exogenous NA on transmitter release in the anococcygeus are in progress and will hopefully provide an unequivocal answer as to which of the two possibilities is the correct one.

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Modulation of noradrenergic transmission by the presynaptic α-inhibitory feedback process in the rat heart

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There is conflicting experimental evidence about the extent to which the presynaptic α-inhibitory process modulates noradrenergic transmission. The findings that phentolamine potentiated the tachycardia to cardiac nerve stimulation in guinea-pig atria (Langer, Adler-Graschinsky & Giorgi, 1977), pithed rats (Doxey, 1977) and anaesthetized dogs (Lokhandwala & Buckley, 1976) suggests that the α-inhibitory feedback process does modulate noradrenergic transmission. However, other investigators failed to demonstrate a potentiating effect of phentolamine in rat isolated atria (Idowa & Zar, 1977) or in anaesthetized dogs (Antonaccio, Halley & Kerwin, 1974; Cavero, Lefèvre & Roach, 1977). These differences might be attributable to the species used, the frequency and duration of stimulation, or the extent to which noradrenaline was inactivated by neuronal re-uptake. In an attempt to analyse the various factors involved, the effects of presynaptic α-adrenoceptor blockade on the tachycardia produced during short (15 s) or prolonged (2–4 min) periods of cardiac nerve stimulation have been re-evaluated in pithed rats, before and after inhibition of uptake$_1$.

The tachycardia produced during short periods of stimulation at 1 Hz was potentiated 10–20% by phentolamine (0.2 mg/kg i.v.) or by cocaine (5 mg/kg i.v.). Cocaine, but not phentolamine, also prolonged the duration of the response. The combination of phentolamine and cocaine produced no greater potentiation of responses at 1 Hz than did either drug alone.

Phentolamine (0.2 mg/kg i.v.) did not significantly enhance responses to prolonged periods of stimulation at 0.1 or 1 Hz; responses at 0.2 and 0.5 Hz were potentiated, but only by 21 and 12% respectively. Cocaine (5 mg/kg i.v.) potentiated responses at 0.1 Hz by 50%, but did not significantly enhance responses to stimulation at 0.2–1 Hz. The combination of phentolamine and cocaine potentiated responses to prolonged stimulation at 0.1 Hz by a further 10–20%, but did not enhance responses at 0.2–1.0 Hz. Similar results were obtained with a higher dose of phentolamine (2.0 mg/kg i.v.) used either alone or in combination with cocaine.

Phentolamine (0.2 mg/kg) reduced the positive chronotropic response to noradrenaline (0.5 μg/kg) and tyramine (50 μg/kg) by 21 and 15% respectively, which shows that the small potentiation by phentolamine of responses to cardiac nerve stimulation is not mediated postsynaptically.

It is concluded that in the rat heart the presynaptic α-adrenoceptor feedback process exerts some control over noradrenergic transmission, but the effect is small and does not seem to be limited by uptake$_1$.